

1 RRH: M. A. Baker et al.

2 LRH: Algal diversity and N uptake

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4 **Differences in nitrate uptake among benthic algal assemblages in a mountain stream**

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25 **Abstract.** We evaluated how benthic algal assemblages that vary in composition, richness, and
26 other diversity metrics remove $\text{NO}_3\text{-N}$ from the water column of a mountain stream. Ecological
27 theory and empirical studies suggest that ecosystem process rates should increase as richness
28 increases because of niche separation or activity of dominant taxa. Accordingly, we predicted
29 that algal assemblages with highest richness would show the highest rates of $\text{NO}_3\text{-N}$ uptake. To
30 test this prediction, we transplanted 225 rocks representing 3 patch types (green, yellow, and
31 brown) that differed macroscopically in algal composition from a lake outflow stream to a lake
32 inflow stream where an experimental release of $^{15}\text{N}\text{-NO}_3$ was ongoing. We measured ^{15}N uptake
33 in each patch type during the stable isotope release. Benthic algal richness varied from 28 genera
34 in the green patch type and 26 genera in the yellow patch type to 22 genera in the brown patch
35 type. Without accounting for differences in chlorophyll *a* content, $\text{NO}_3\text{-N}$ uptake ($2.1\text{--}3.3 \times 10^{-4}$
36 d^{-1}) was highest in the green patch type, lowest ($0.3\text{--}0.6 \times 10^{-4}/\text{d}$) in the yellow patch type, and
37 intermediate ($1.2\text{--}1.5 \times 10^{-4}/\text{d}$) in the brown patch type. $\text{NO}_3\text{-N}$ uptake normalized to chlorophyll
38 *a* increased in concert with algal richness in the 3 patch types. This result supports the hypothesis
39 that increased assemblage diversity leads to higher rates of community processes. Aside from
40 diversity differences per se, lower rates of $\text{NO}_3\text{-N}$ uptake in the brown patch type might be the
41 consequence of differences in functional characteristics of the taxa present. Approximately 29%
42 of algal biovolume in the brown patch type consisted of taxa capable of N_2 -fixation, a result that
43 suggests that algae in this patch type might be capable of meeting N needs via N_2 -fixation rather
44 than via removal from the water column.

45

46 **Key words:** periphyton, nutrient immobilization, nitrogen-15, stable isotope, diversity.

47 Study of the relationship between biodiversity and ecosystem functioning has a long
48 tradition in ecology, and stems from work on diversity and stability in the context of animal
49 populations and energy flow through food webs (MacArthur 1955). Recent research and debate
50 have centered on relationships between productivity and diversity of terrestrial plant
51 communities (e.g., Tilman 1999), nutrient cycling and terrestrial plant diversity (e.g., Hooper and
52 Vitousek 1997), and decomposition and decomposer diversity in terrestrial (e.g., Naeem et al.
53 1995) and aquatic (e.g., Jonsson and Malmqvist 2000) ecosystems. A variety of patterns and
54 mechanisms relating biodiversity to ecosystem functioning have been reported, and the recent
55 consensus is that richness is important, but functional characteristics of taxa strongly influence
56 ecosystem properties (Hooper et al. 2005).

57 Benthic algae play a vital role in immobilization of nutrients from the water column of
58 streams (Fisher et al. 1982, Mulholland 1992, Covich et al. 2004) and contribute to stream self-
59 purification as an ecosystem service upon which human society depends (Hooper et al. 2005).
60 Much recent research in stream ecosystem ecology has focused on quantifying rates of stream
61 nutrient uptake across biomes (Peterson et al. 2001, Mulholland et al. 2008), across land uses
62 (Bernot et al. 2006, O'Brien et al. 2007), and across time (Simon et al. 2005, Hoellein et al.
63 2007). Physical factors such as groundwater–surface water exchange (Valett et al. 1996),
64 discharge (Peterson et al. 2001, Wollheim et al. 2001), hydrogeomorphology (Doyle et al. 2003),
65 temperature (Martí and Sabater 1996), and nutrient concentration (Bernot et al. 2006) control the
66 rate of nutrient uptake in streams. Fewer studies have examined biological factors that control
67 nutrient uptake, but nutrient uptake rates are positively correlated with rates of ecosystem
68 metabolism (Hall and Tank 2003, Gucker and Pusch 2006, Mulholland et al. 2006).

69 Human activities are altering the biological composition of communities world wide

70 (Vitousek et al. 1997), and stream benthic algal assemblages are no exception. Diatoms, in
71 particular, are responsive to anthropogenic nutrient inputs (Pan et al. 2000) and are used as
72 indicators of water quality (Stevenson 1998). Algal assemblages also are susceptible to
73 invasions. For example, *Didymosphenia geminata*, a diatom native to oligotrophic waters of the
74 Northern Hemisphere, has greatly expanded its range and caused nuisance blooms and changes
75 to stream macroinvertebrate grazer assemblages in New Zealand and elsewhere (Kilroy 2004).

76 Little is known about the functional role of specific algal taxa or the effects of algal
77 community structure (composition, richness, diversity) on nutrient uptake in streams (Covich et
78 al. 2004). N₂-fixing taxa might increase in abundance as water-column N declines (Fisher et al.
79 1982). Mulholland et al. (1991) showed that nutrient reductions and herbivory by snails changed
80 periphyton community composition and increased P cycling rates. Other studies have
81 documented little change in algal assemblages but higher rates of production and nutrient cycling
82 in response to nutrient enrichment (Miller et al. 1992, Peterson et al. 1993).

83 Strong inferences relating biodiversity to ecosystem functioning have been made using
84 theoretical (Loreau 2000) and empirical approaches (Tilman 1999) and indicate that increased
85 richness might lead to asymptotic increases in the magnitude of ecosystem processes over short
86 time scales. Our objective was to examine how different benthic algal assemblages remove NO₃-
87 N from the water column of a mountain stream during a stable isotope (¹⁵N) tracer test. We
88 hypothesized that assemblages with higher richness would exhibit higher rates of ¹⁵N uptake
89 because assemblages with higher diversity are more likely to include a dominant taxon (i.e.,
90 sampling effect, sensu Tilman 1997) responsible for much of the processing rate, or are more
91 likely to include a group of taxa whose functional traits are complementary (sensu Loreau 2000)
92 and reduce interspecific competition, thereby leading to higher processing rates.

93

94

Methods

95 *Study site*

96 We worked in the Bull Trout Lake watershed in the Boise National Forest in central
97 Idaho, USA. Bull Trout Lake (44° N, 115° W) is a 0.28-km² dimictic lake formed by a moraine
98 dam. Spring Creek and Warm Springs Creek are the 2nd-order streams that flow into and out of
99 the lake, respectively (Fig. 1). Catchment area at Warm Springs Creek is 11.7 km² and elevation
100 is 2118 m above sea level. Both streams have an open canopy with vegetation dominated by
101 willows (*Salix* sp.), sedges (*Carex* sp.), and grasses along the stream banks and lodgepole pine
102 (*Pinus contorta*) in forested uplands (Arp et al. 2006).

103 Physicochemical variables differ greatly in Spring Creek (lake inlet) and Warm Springs
104 Creek (lake outlet). During our study (August 2003), NO₃-N averaged 6.9 µg/L in the inlet and
105 2.5 µg/L in the outlet. Total N typically shows the opposite pattern during baseflow with
106 concentrations on the order of 20 to 30 µg/L in the inlet and 90 to 100 µg/L in the outlet (Arp and
107 Baker 2007, Marcarelli and Wurtsbaugh 2007). Outlet water temperatures are, on average, 10°C
108 warmer than inlet water temperatures (Arp and Baker 2007, Marcarelli and Wurtsbaugh 2007).

109 Rock substrates in the lake outlet are larger (84th percentile particle diameter [D₈₄] = 39
110 mm) compared to those in the lake inlet (D₈₄ = 18 mm) because of sediment trapping by the lake
111 and absence of tributaries below the lake to supply new sediment (Arp et al. 2007). Periphyton
112 biomass in the outlet stream often is colimited by N and P during summer months (Marcarelli
113 and Wurtsbaugh 2007). Distinct patch types that differ macroscopically in benthic algal
114 composition are observed in Warm Springs Creek (the outlet), but not in Spring Creek (the inlet).
115 Hereafter, we refer to these patch types as *green*—characterized by green filamentous algae,

116 *brown*—characterized by brown flocs of periphyton, and *yellow*—characterized by a yellow, less
117 developed (thin) periphyton. Rocks from each patch type were transplanted to the inflow stream
118 before the experiment (see *Experimental design* below). At the time of the experiment, stream
119 flow in the inlet at the station above the lake ranged from 120 to 150 L/s with a mean velocity of
120 0.26 m/s (Arp et al. 2006, 2007).

121

122 *Algal assemblage analysis*

123 We collected 5 rocks from each patch type for analysis of algal assemblage composition.
124 We scrubbed the periphyton from rock surfaces with a soft-bristled brush, collected it
125 volumetrically, and pooled it by patch type. We measured rock surface area by covering the rock
126 surfaces with aluminum foil and relating the mass of foil to known surface area (Bergey and
127 Getty 2006). We preserved a 60-mL homogenized aliquot of slurry from each patch type with
128 Lugol's solution (final concentration 1%) and sent the aliquots to PhycoTech (St. Joseph,
129 Michigan) for periphyton analysis. There, samples were mounted in triplicate using 2-
130 hydroxypropyl methacrylate (Crumpton 1987) and examined by epifluorescence microscopy to
131 identify and enumerate algal taxa to genus. A minimum of 300 natural units and 15 fields at
132 200× magnification were counted except when samples were dominated by diatoms, in which
133 case a minimum of 400 natural units and 15 fields at 1000× magnification were counted.
134 Biovolume was calculated using Phycotech's proprietary software, Aquatic Sample Analysis,
135 from measurements of greatest axial length dimension, length, depth, and width of cells for 10 to
136 30 natural units of each taxon. Biovolume provides a better estimate of algal biomass than do
137 total counts, so we report only biovolume data; we observed similar patterns with cell counts.
138 We quantified algal diversity of each patch type by calculating richness, evenness,

139 Shannon–Weiner index (H'), and Bray–Curtis similarity in Systat V.5 (SPSS, Chicago, Illinois)
140 for the genera identified. We chose to quantify diversity as richness, evenness, and H' because
141 many studies relating diversity to ecosystem functioning have used these metrics, and we wanted
142 our data to be comparable to those studies. We used Bray–Curtis similarity for pair-wise
143 comparison of patch composition.

144

145 *Experimental design*

146 We took advantage of a ^{15}N tracer study to evaluate how the algal assemblages from each
147 patch type immobilize N from the water column. Briefly, a solution containing 75 g of ^{15}N (as
148 27% $\text{Na}^{15}\text{NO}_3$) was administered to Springs Creek at a rate of 10.4 mL/min for 14 d beginning 5
149 August 2003 at a site ~1895 m above Bull Trout Lake (Fig. 1). The tracer test elevated the
150 isotopic signature of the NO_3^- pool to 11,670‰ above background on average, with higher
151 values at the top of the reach and lower values at the bottom of the reach. Changes in ambient
152 $\text{NO}_3\text{-N}$ concentration as a result of the tracer test were not detectable (Hall, R. O., University of
153 Wyoming, unpublished data).

154 We transplanted 75 rocks (median particle size $[\text{D}_{50}]\text{--}\text{D}_{84} = 16\text{--}39$ mm; Arp et al. 2007)
155 from each patch type from Warm Springs Creek (lake outlet) to Springs Creek (lake inlet) 3 d
156 before the isotope experiment commenced. We arranged 15 rocks from each patch type in plastic
157 baskets (18 cm \times 26 cm) and placed the buckets in riffles at 5 sites: 62, 120, 1069, 1560, and
158 1890 m downstream of the ^{15}N -drip site (Fig. 1).

159 We collected periphyton samples from each patch type at each location (pooled from 5
160 rocks) the day before the ^{15}N -release and 8 and 14 d after the release began. We collected
161 periphyton and measured rock area as described above. We filtered homogenized aliquots of the

162 slurry onto 4 precombusted glass-fiber filters (Whatman GF/F, Whatman International Ltd.,
 163 Maidstone, England UK). We folded 2 of the filters in aluminum foil and froze them before
 164 extraction with 95% ethanol for analysis of chlorophyll *a* (Welschmeyer 1994). We dried the
 165 other 2 filters at 60°C and encapsulated them in tin capsules (Elemental Micoranalysis Ltd.,
 166 Manchester, Massachussets) for measurement of isotopic composition and N content
 167 (Mulholland et al. 2000). N content and isotopic enrichment require a target mass of 100 µg N
 168 (D. Harris, University of California, Davis, personal communication), so for samples with large
 169 biomass, we cut the periphyton filters in half prior to encapsulation. We sent all samples to the
 170 University of California (UC) Davis Stable Isotope Facility (Davis, California) for analysis.
 171 There, N content and isotopic composition were measured using a PDZ Europa ANCA-GSL
 172 elemental analyzer connected to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon
 173 Ltd., Cheshire, UK). These instruments combust the samples at 1020°C in a reactor filled with
 174 Cr and Co oxides. N is separated from other gases using a Carbosieve GC column (Supelco,
 175 Bellefonte, Pennsylvania) before quantification (D. Harris, University of California, Davis,
 176 personal communication). Data are reported by this laboratory as the atomic ratio excess
 177 ($AR_{\text{periphyton}}; {}^{15}\text{N}/{}^{14}\text{N} + {}^{15}\text{N}$) and N mass ($N_{\text{periphyton}}; \mu\text{g}$). Values of N and chlorophyll *a* in
 178 periphyton were expressed in units per area of rock sampled (Bergey and Getty 2006). Isotopic
 179 content of periphyton biomass (${}^{15}\text{N}_{\text{periphyton}}$) was calculated from $AR_{\text{periphyton}}$ (Mulholland et al.
 180 2000, 2004) as

$$181 \quad {}^{15}\text{N}_{\text{periphyton}} (\text{mg}/\text{m}^2) = AR_{\text{periphyton}} \times N_{\text{periphyton}} (\text{mg}/\text{m}^2). \quad [1]$$

182 We measured $\text{NO}_3\text{-N}$ concentration using ion chromatography (DIONEX, Sunnyvale,
 183 California) from filtered (Whatman GF/F) samples collected at each location on each sample
 184 date. We measured dissolved ${}^{15}\text{N-NO}_3$ in the water column from 3-L samples collected and

185 filtered (Whatman GF/F) at each stream location on each sample date. We processed these
 186 samples in the laboratory according to protocols outlined in Mulholland et al. (2004). Briefly, we
 187 elevated the pH of samples with MgO to drive off NH₃, concentrated samples to ~100 mL by
 188 boiling, after which we converted the NO₃⁻ in the sample to NH₄⁺ using Devarda's alloy. We
 189 converted this NH₄⁺ to NH₃ with MgO and collected the NH₃ on an acidified filter by diffusion
 190 (Mulholland et al. 2004). The ¹⁵N content on the filter was measured using a Europa Hydra 20/20
 191 mass spectrometer at the UC Davis Stable Isotope Facility as described above. The atomic ratio
 192 excess of ¹⁵N in water samples (AR_{water}) reported by the isotope laboratory was used with
 193 measured NO₃-N concentration (μg/L) to calculate ¹⁵N concentration in the water column as
 194
$$^{15}\text{N}_{\text{water}} (\mu\text{g/L}) = [\text{AR}_{\text{water}} \times \text{NO}_3\text{-N}]_{\text{day 8 or 14}} - [\text{AR}_{\text{water}} \times \text{NO}_3\text{-N}]_{\text{background}}. \quad [2]$$

195 Concentrations on day 8 or 14 are corrected for isotopic content in background samples.

196

197 *Uptake calculations*

198 We calculated immobilization (uptake) of ¹⁵N tracer by each patch type as the
 199 background-corrected ¹⁵N_{periphyton} (mg/m²) incubated at each sample location divided by the
 200 background-corrected ¹⁵N (AR_{water}) in the overlying water column at the same location and the
 201 number of days of exposure to the tracer (8 or 14 d) (Mulholland et al. 2000, 2004) as

$$202 \quad ^{15}\text{N-NO}_3 \text{ uptake (mg m}^{-2} \text{ d}^{-1}) = ^{15}\text{N}_{\text{periphyton}} / [\text{AR}_{\text{water}} \times \text{days of experiment}]. \quad [3]$$

203 This calculation assumes that the periphyton has reached equilibrium with tracer in the water
 204 column at the time of sampling (Mulholland et al. 2000). We averaged values from each site to
 205 estimate mean uptake rates by each patch type for each sample date. To account for differences
 206 in N content of each patch type, we calculated N-specific uptake (units are 1/d) by dividing the
 207 areal ¹⁵N-NO₃ uptake (mg m⁻² d⁻¹) above by the areal N content (mg/m²) of each patch type

208 (Dodds et al. 2004). Hereafter, we report results as N-specific uptake (NO₃-N uptake).

209

210 *Hypothesis tests*

211 We compared NO₃-N uptake rates with 2-way analysis of variance (ANOVA) with patch
212 type and day of experiment as factors. We followed significant ANOVAs by Tukey–Kramer
213 Honestly Significant Difference tests (SAS, version 8; SAS Institute, Cary, North Carolina). We
214 considered rates significantly different at $p < 0.05$. We used linear regression to assess whether
215 differences among rates were related to chlorophyll-*a* content (SAS version 8). Our study had
216 only 3 levels of diversity, so we did not use statistics to test hypotheses for relationships between
217 richness and NO₃-N uptake.

218

219 **Results**

220 *Algal assemblage composition*

221 Algal assemblage richness differed among patch types, with 28 genera in the green patch
222 type, 26 genera in the yellow patch type, and 22 genera in the brown patch type (Table 1, Fig. 2).
223 Six taxa were unique to the green patch, whereas 4 and 3 taxa were unique to the yellow and
224 brown patches, respectively (Table 1).

225 The algal assemblage from the green patch type was dominated by the Chlorophyta
226 *Spirogyra* and *Rhizoclonium*, which represented >90% of total biovolume. The algal assemblage
227 from the yellow patch type was also dominated by Chlorophyta (85% of biovolume); *Spirogyra*
228 accounted for 61% of biovolume and *Bulbochaete* accounted for 25% of biovolume. In contrast,
229 the periphyton assemblage from brown patches was dominated by Bacillariophyta (>82% of
230 biovolume); *Synedra*, *Cymbella*, *Fragilaria*, and *Epithemia* each accounted for ~10% of

231 biovolume. Cyanophyta also were important (15% of biovolume) in the brown patch type.

232 The green and yellow patch types had higher algal richness than did the brown patch
233 type, but the brown patch type had higher evenness (Table 2, Fig. 2) and higher H' than the
234 green or yellow patch types. Bray–Curtis distances indicated that the brown patch type had the
235 most unique algal composition, whereas the yellow and green patch types were most similar to
236 each other (Table 2).

237

238 *¹⁵N-nitrate uptake rates*

239 NO₃-N uptake rates differed significantly among patch types (ANOVA, $df = 3$, $F =$
240 36.09 , $p < 0.001$; Fig. 3). NO₃-N uptake rates were highest in the green patch type, intermediate
241 in the brown patch type, and lowest in the yellow patch type (Fig. 3). NO₃-N uptake rates did not
242 differ between sample days, except in the green patch type, where NO₃-N uptake was
243 significantly higher on day 14 than on day 8 (Fig. 3).

244 NO₃-N uptake rate was not significantly correlated with chlorophyll *a* content ($R^2 = 0.40$,
245 $p > 0.05$; Fig. 4). Algae from the yellow patch type had 3× lower chlorophyll *a* concentrations (~
246 20 mg/m^2) than did algae from the brown and green patch types (~ 60 mg/m^2) (ANOVA, $df = 3$,
247 $F = 36.09$, $p < 0.01$), a result that explained some portion of the difference in NO₃-N uptake by
248 the yellow patch.

249 NO₃-N uptake rates did not increase in concert with algal richness, as predicted from the
250 diversity–function hypothesis. NO₃-N uptake was greatest for the green patch type and lowest for
251 the yellow patch type (Figs 3, 4). However, when normalized to chlorophyll *a* content, NO₃-N
252 uptake rate increased in concert with richness, as predicted by the diversity–function hypothesis
253 (Fig. 5). Algal composition also was related to NO₃-N uptake rate, which was negatively related

254 to Cyanophyta biovolume and positively related to Chlorophyta biovolume (Fig. 5).

255

256

Discussion

Relationship between diversity and ecosystem functioning

258 Our study is the 1st attempt to use a stable isotope experiment to relate algal diversity to

259 ecosystem functioning in streams. As average richness increased, chlorophyll-corrected NO₃-N

260 uptake appeared to increase in concert. However, our results should be interpreted with great

261 caution because we had only 3 levels of richness that differed by only 6 genera. We chose to

262 identify taxa to the genus level because of financial constraints, but positive correlation between

263 genus richness and species richness has been reported for stream periphyton (Hill et al. 2001).

264 We also did not separate algal from bacterial and fungal components of the periphyton, and this

265 decision might bias our results and interpretation. Nevertheless, the pattern we observed is

266 consistent with that seen in studies from a variety of ecosystems, including grasslands (Tilman

267 1999) and other terrestrial ecosystems (Hooper et al. 2005), estuaries (Zedler et al. 2001), and

268 stream filter-feeding guilds (Cardinale et al. 2002).

269 Our results oppose predictions from the diversity–function hypothesis. The algal

270 assemblage in the brown patch type had the highest values of H' and evenness and was most

271 different in composition from the algal assemblage in the other patch types as measured by Bray-

272 Curtis distance, yet the algal assemblage in the brown patch type had the lowest NO₃-N uptake

273 rate when normalized to chlorophyll *a*. Low NO₃-N uptake in the brown patch type might be

274 related to differences in functional characteristics of the dominant taxa in each patch, rather than

275 to diversity per se. Algae in the brown patch included a relatively high proportion of taxa

276 potentially capable of N₂-fixation (Table 1). These taxa included Cyanophyta (Gallon 2004),

277 which accounted for ~15% of biovolume, and diatoms (*Epithemia* and *Rhopalodia*) with N₂-
278 fixing endosymbionts (Prechtel et al. 2004), which accounted for another 14% of biovolume. In
279 contrast, taxa potentially capable of N₂-fixation accounted for ~5% of biovolume in the yellow
280 patch type and <0.03% of biovolume in the green patch type. The negative relationship between
281 NO₃-N uptake and biovolume of Cyanophyta supports this interpretation. Algae from brown
282 patches might be able to meet its N demands via N₂-fixation rather than via assimilation of N
283 from the water column. Rates of N₂-fixation on the order of 8.7 μg N m⁻² h⁻¹ have been
284 measured in this system (Marcarelli et al. 2008), and N₂-fixation rates are correlated with
285 presence of N₂-fixing taxa (Marcarelli and Wurtsbaugh 2006).

286 Stable isotopes have been used in terrestrial ecosystems to examine relationships between
287 diversity and ecosystem functioning. For example, Hooper and Vitousek (1997) used ¹⁵N-tracers
288 to show that productivity and N retention in a Mediterranean grassland were best explained by
289 community composition rather than richness. Hooper and Vitousek (1997) also explained their
290 results by suggesting that functional characteristics of the taxa better explained ecosystem
291 process rates than did richness or other measures of diversity.

292

293 *Autecological factors influencing our results*

294 Substratum size can influence periphyton biomass and assemblage composition directly if
295 large rocks are more stable and less susceptible to disturbance than are small rocks (Biggs 1996).
296 The differences we present here probably are not a result of substratum size because we used
297 particles in the D₅₀–D₈₄ range. Average surface area of rocks sampled was smallest for the green
298 patch type (122 cm²), and greatest for the yellow patch type (187 cm²). Chlorophyll *a* was
299 highest in the green patch type and lowest in the yellow patch type.

300 Physiognomic differences among algae can affect nutrient immobilization because
301 growth form and stature affect resource (light and nutrient) supply to algal mats (Stevenson
302 1996). Competitively dominant diatoms in algal mats are those that produce mucilaginous stalks,
303 which enable better access to light and nutrients (McCormick and Stevenson 1991).
304 Mucilaginous stalks were visible in the brown patch type, but increased resource supply does not
305 explain low NO₃-N uptake by periphyton in this patch type. If nutrient delivery rates were
306 higher, this patch should have assimilated more N than we measured.

307 That NO₃-N uptake was not linearly correlated with chlorophyll *a* is interesting because
308 the pigment is rich in N and usually is a good estimate of N requirements of algae (Graham and
309 Wilcox 2000). However, the relationship probably does not hold in situations in which algal
310 assemblages are distinctly different because algae in the brown patch type had low NO₃-N
311 uptake relative to its internal store of chlorophyll *a*.

312 We do not know the degree to which each genus contributed to NO₃-N uptake. Some taxa
313 (*Oocystis*, *Tetraedron*, and *Pediastrum*) are more typical of lake phytoplankton than of stream
314 periphyton (Wehr and Sheath 2003) and might be cells that were trapped in the periphyton when
315 the rocks were in the lake outflow. These taxa were present in low numbers and probably did not
316 contribute significantly to NO₃-N uptake. Dominant taxa, such as *Spirogyra* in the green and
317 yellow patch types, probably contributed significantly to NO₃-N uptake. *Spirogyra fluviatum* has
318 high N requirements, particularly at high flows (Borchardt 1994).

319 Taxa differ in their requirements for N and their affinity for NO₃-N, but NO₃-N uptake
320 probably was not saturated in our study because ambient NO₃-N concentration was <10 µgN/L
321 and did not change as a result of the tracer test. Many of the genera we found (e.g., *Mougeotia*,
322 *Consmarium*, *Teilingia*) are common in oligotrophic waters (Wehr and Sheath 2003) and have

323 tolerance values for total N between 26 and 140 $\mu\text{gN/L}$ (Lowe and Pan 1996). Concentrations
324 $<100 \mu\text{gN/L}$ are growth limiting for benthic algae grown in artificial streams (Lohman et al.
325 1991). $\text{NO}_3\text{-N}$ uptake by *Cladophora glomerata*, a species common in nutrient-rich water,
326 saturates at concentrations $\sim 330 \mu\text{gN/L}$ (Lohman and Priscu 1992).

327

328 *Implications for studies of nutrient uptake*

329 Our results have interesting implications for studies of nutrient cycling in streams. First,
330 nutrient uptake rates vary widely among patch types, even when they are in close proximity (cm)
331 to each other. This result reinforces the notion that care should be taken during sampling to
332 stratify for habitat or patch type to capture the variability inherent within the stream reach being
333 studied (Mulholland et al. 2000).

334 Second, nutrient enrichment decreases algal diversity in benthic (e.g., Whitton et al.
335 1991) and pelagic (e.g., Schindler 1977) systems. Our study might provide some insight to why
336 nutrient uptake rates decline as nutrient availability increases. This pattern often is attributed to
337 nutrient saturation (e.g., Earl et al. 2006), which might be the case when nutrient uptake is
338 measured experimentally with sequential releases of nutrient over short time scales (h).

339 However, when this pattern is observed across streams that differ in nutrient availability (e.g.,
340 O'Brien et al. 2007), changes in assemblage structure and diversity should not be ruled out as
341 possible causes.

342 Streams are important landscape features in the transport and storage of nutrients
343 (Peterson et al. 2001), but little is known about how benthic algal assemblages contribute to
344 nutrient transport and storage. Our results illustrate that algal assemblage composition might
345 affect nutrient uptake rate. Future studies of factors regulating nutrient uptake in streams would

346 benefit from including an autecological approach with the synecological approach already
347 inherent in the research. The stable isotope approach we present here might be one way to do so.
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Figure Captions

Fig. 1. Study site in central Idaho, USA, showing original location of rocks and locations to which they were transplanted and during the $^{15}\text{N}\text{-NO}_3$ experiment.

Fig. 2. Rank abundance curves (abundance rank 1 is highest by biovolume) for periphyton assemblages from 3 patch types.

Fig. 3. Mean (± 1 SE) $\text{NO}_3\text{-N}$ uptake rates for periphyton assemblages from 3 patch types on days 8 and 14 of $^{15}\text{N}\text{-NO}_3$ release. Bars with the same letter are not significantly different (analysis of variance followed by Tukey-Kramer post hoc comparisons $p < 0.05$).

Fig. 4. Relationship between mean (± 1 SE) periphyton chlorophyll *a* content and mean (± 1 SE) $\text{NO}_3\text{-N}$ uptake for each patch type on days 8 and 14 of $^{15}\text{N}\text{-NO}_3$ release.

Fig. 5. Relationship between N-uptake (normalized to chlorophyll *a*) and % Cyanophyta biovolume (closed symbols) and % Chlorophyta biovolume (open symbols) in 3 patch types with different algal richness.

541 Table 1. Periphyton taxa list by patch type in order of abundance by biovolume. Taxa unique to
 542 each patch type are indicated in bold font.

Green patch	Yellow patch	Brown patch
<i>Spirogyra</i>	<i>Spirogyra</i>	<i>Synedra</i>
<i>Rhizoclonium</i>	<i>Bulbochaete</i>	<i>Cymbella</i>
<i>Synedra</i>	<i>Calothrix</i> ⁺	<i>Fragilaria</i>
<i>Fragilaria</i>	<i>Protoderma</i>	<i>Epithemia</i> ^b
<i>Cymbella</i>	<i>Achnanthes</i>	<i>Calothrix</i> ^a
<i>Achnanthes</i>	<i>Synedra</i>	<i>Oscillatoria</i> ^a
<i>Gomphonema</i>	<i>Rhopalodia</i> ^b	<i>Achnanthes</i>
<i>Navicula</i>	<i>Fragilaria</i>	<i>Eunotia</i>
<i>Stigeoclonium</i>	<i>Gomphonema</i>	<i>Rhopalodia</i> ^b
<i>Mougeotia</i>	<i>Cymbella</i>	<i>Navicula</i>
<i>Aulacoseira</i>	<i>Closterium</i>	<i>Teilingia</i>
<i>Bulbochaete</i>	<i>Synechocystis</i> ^a	<i>Gomphonema</i>
<i>Cocconeis</i>	<i>Navicula</i>	<i>Anomoeneis</i>
<i>Pseudanabaena</i> ^a	<i>Cocconeis</i>	<i>Nitzschia</i>
<i>Synechocystis</i> ^a	<i>Stigeoclonium</i>	<i>Oocystis</i>
<i>Tabellaria</i>	<i>Eunotia</i>	<i>Cocconeis</i>
<i>Oedogonium</i>	<i>Denticula</i>	<i>Euastrum</i>
<i>Anomoeneis</i>	<i>Anomoeneis</i>	<i>Synechocystis</i> ^a
<i>Chlorococcum</i>	<i>Chlorococcum</i>	<i>Pediastrum</i>
<i>Scenedesmus</i>	<i>Diploneis</i>	<i>Scenedesmus</i>

<i>Epithemia</i> *	<i>Oscillatoria</i> ^a	Chlorococcaceae ^c
<i>Cosmarium</i>	<i>Cosmarium</i>	<i>Protoderma</i>
<i>Protoderma</i>	<i>Nitzschia</i>	
<i>Eunotia</i>	<i>Aphanocapsa</i> ^a	
<i>Tetraedron</i>	<i>Aulacoseira</i>	
<i>Nitzschia</i>	<i>Scenedesmus</i>	
<i>Oscillatoria</i> ^a		
<i>Oocystis</i>		

- 543 ^a Division Cyanophyta
- 544 ^b Diatoms with endosymbiont Cyanobacteria
- 545 ^c Identification to family
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547 Table 2. Algal diversity metrics calculated for each patch type.
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	Green patch	Yellow patch	Brown patch
Richness	28	26	22
Shannon–Weiner	0.814	1.33	2.25
Shannon evenness	0.244	0.375	0.718
Bray–Curtis distance	Green vs yellow	Yellow vs brown	Brown vs green
	0.496	0.611	0.621

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